PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

PLOUGHMAN, VINGTOFT & PARTNERS A/S
Sankt Ann Plads 11
P.O. Box 3007
DK-1021 Copenhagen K
DANEMARK

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	19.09.00
Applicant's or agent's file reference: 21134 PC 1	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK99/00382	02/07/1999	03/07/1998

Applicant
CHR. HANSEN A/S et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Vullo, C Tel.+49 89 2399-8061

Form PCT/IPEA/416 (July 1992)

F..TENT COOPERATION TREA..

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
21134 PC 1		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK99/00382	02/07/1999	03/07/1998
International Patent Classification (IPC) or national classification and IPC C12N1/20		
Applicant CHR. HANSEN A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report
   II ☐ Priority
   III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   IV ☐ Lack of unity of invention
   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   VI ☐ Certain documents cited
   VII ☐ Certain defects in the international application
   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17/12/1999	19.09.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Heimann-Pohl, B Telephone No. +49 89 2399 8713

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK99/00382

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-25	as originally filed			

Claims, No.:

1-27	as received on	13/07/2000	with letter of	10/07/2000

Drawings, sheets:

1/12-12/12	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-27
	No:	Claims
Inventive step (IS)	Yes:	Claims 1-27
	No:	Claims
Industrial applicability (IA)	Yes:	Claims 1-27
	No:	Claims

2. Citations and explanations

see separate sheet

1). The present application relates to a method of preventing bacteriophage infection of bacterial cultures due to a mutation which renders the bacteria incapable of DNA replication, RNA transcription or protein synthesis, but which retain their capability of being metabolically active. The application further relates to the so defined bacteria and their use in the production of edible products.

2). Novelty, Inventive Step and Industrial Applicability (Box V)

2.1). Prior Art

D1: RICHARDSON G H ET AL: 'Proteinase negative variants of Streptococcus cremoris for cheese starters' JOURNAL OF DAIRY SCIENCE, vol. 66, 1983, pages 2278-2286, XP002082743 cited in the application
D2: NILSSON D & LAURIDSEN A A: 'Isolation of purine auxotrophic mutants of Lactococcus lactis and characterization of the gene hpt encoding hypoxanthine guanine phosphoribosyltransferase.' MOLECULAR AND GENERAL GENETICS, vol. 235, 1992, pages 359-364, XP002032525 cited in the application

D1 discloses proteinase negative variants of Streptococcus cremoris which are able to acidify milk. However, these variants are still growing, although at a lower rate than the proteinase positive strain, as stated on page 2278 "Generation times of proteinase negative cells were 3.5 times as long in cheese milk as those of proteinase positive, suggesting a probable reduction of bacteriophage and antibiotic problems."

D2 discloses the purine auxotrophic mutant DN105 of L. lactis (paragraph spanning page 360, right col.-page 361, left col.). D2 does not refer to the use of said mutant in food production.

2.2). Novelty

Growth, as in the case of D1, requires DNA replication, RNA transcription and protein synthesis, therefore variants of D1 do not appear to fall under the requirements set out in claim 1, 2, 3, 8-11 and 26. Also the subject matter of

claims 4-7, 12-25 and 27 appears to be novel with regard to D1.
The strains disclosed in D2 were not used in the methods of claims 1-17 and 24-27. Also the subject matter of claims 20-23 appears to be novel with regard to D2.
The mutants DN101-104 which are also disclosed in D2 are disclaimed in claim 18 and 19, therefore said claims can be regarded as novel (Art. 33 (2) PCT).

2.3). Inventive Step

The problem underlying the present application is that bacterial cultures, used for example for the fermentation of milk, are often subject to bacteriophage infection. This problem is solved by the provision of bacteria that are purine or pyrimidine mutants but still capable of metabolizing substrate material.
It is common knowledge that DNA replication, RNA transcription and protein synthesis is necessary for bacteriophage development.
However, with regard to the description page 17, lines 11-13, it was apparently not known that purine auxotrophic mutants of L.lactis not growing in milk would be capable of acidifying such a substrate material.
Therefore the use of a purine auxotrophic mutant of L.lactis in the method of claims 1-4, 6-11and 27 involves an inventive step (overcoming of a prejudice).

The use of a pyrimidine (Pyr$^-$) mutant is not obviously derivable from the prior art. Therefore claims 5 and 20-27 are inventive.

In the method of claims 12-14 the strain not being susceptible to attack by bacteriophages due to not being capable of DNA replication, RNA transcription or protein synthesis is, additionally, genetically modified such that it has an enhanced metabolic activity relative to its parent strain. Although not reduced into practice in the present application, it seems likely that the skilled person would be able to obtain such a strain with regard to the teaching of example 2 (page 18 of the present application) in combination with page 11 and the teaching of WO 98/10089.
In the method of claims 15 and 16 the feature "not being capable of DNA replication, RNA transcription or protein synthesis" should be due to a conditional mutation. Although not reduced into practice in the present application, it seems nevertheless likely that the skilled person would be able to obtain such a strain

using his or her general knowledge of methods to use to provide such a conditional mutant.

In the method of claims 17 a strain which is not capable of DNA replication is unable to carry out mitosis, but it would still be able of growing in size, since, under this condition synthesis of new enzymes is not affected.

Thus also the methods of claims 12-17 can be considered to involve an inventive step.

2.4). Industrial Applicability

The subject matter of claims 1-27 appears to be industrial applicable.

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Chr. Hansen A/S
International Patent Application No. PCT/DK99/00382
Publication No. WO 00/01799

AMENDED CLAIMS, July 2000

1. A method of modifying a substrate material by means of a bacterial culture which is capable of being metabolically active in said substrate, whereby the bacterial culture is not susceptible to attack by bacteriophages, the method comprising

(i) isolating a bacterial strain which is not capable of DNA replication, RNA transcription or protein synthesis in said substrate material but is capable of metabolically modifying the substrate material,

(ii) propagating the selected strain in a medium wherein the strain is capable of replicating to obtain a culture of said strain,

(iii) adding the thus obtained bacterial culture to the substrate material and keeping the material under conditions where the culture is metabolically active.

whereby, if the substrate material is contaminated with a bacteriophage, the metabolic activity of the bacterial culture is substantially unaffected by the bacteriophage.

2. A method according to claim 1 wherein the substrate material is limited with respect to at least one compound that is required by the bacterial strain for DNA replication, RNA transcription or protein synthesis.

3. A method according to claim 2 wherein the bacterial strain is a mutant strain being auxothrophic in respect of a compound which is not present in the substrate material and which is required by the strain for replication.

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4. A method according to claim 3 wherein the mutant strain is a Pur⁻ mutant including *Lactococcus lactis* strain DN105 deposited under the accession number DSM 12289.

5. A method according to claim 3 wherein the mutant strain is a *thyA* mutant including *Lactococcus lactis* strain MBP71 deposited under the accession number DSM12891.

6. A method according to any of claims 2 to 5 wherein the strain in said substrate material is not capable of performing at least one activity selected from the group consisting of DNA replication, RNA transcription and protein synthesis.

7. A method according to claim 1 wherein the substrate material contains at least one compound that inhibits the DNA replication, RNA transcription or the protein synthesis of the bacterial strain.

8. A method according to claim 1 wherein the substrate material is a starting material for an edible product, the material is selected from the group consisting of milk, a vegetable material, a meat product, a must, a fruit juice, a wine, a dough and a batter.

9. A method according to claim 1 wherein the bacterial culture is selected from the group consisting of *Lactococcus* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Pediococcus* spp., *Streptococcus* spp., *Propionibacterium* spp., *Bifidobacterium* spp., *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp., *Enterobacteriaceae* spp. including *E. coli*, *Actinomycetes* spp., *Corynebacterium* spp. and *Brevibacterium* spp.

10. A method according to claim 9 wherein the bacterial culture is of *Lactococcus lactis*.

11. A method according to claim 1 wherein the bacterial strain is added to the substrate material at a concentration in the range of 10^5 to 10^9 CFU/ml or g of the material.

12. A method according to claim 1 wherein the culture comprises a genetically modified strain which, relative to its parent strain is enhanced in at least one metabolic pathway.

13. A method according to claim 12 wherein the genetically modified strain has, relative to its parent strain, an enhanced metabolic activity selected from the group consisting of enhanced glycolytic flux and enhanced flux through the pentose phosphate pathway.

14. A method according to claim 13 wherein the genetically modified strain has, relative to its parent strain, an enhanced ATPase activity.

15. A method according to claim 1 wherein the bacterial culture comprises a strain which is a conditional mutant which at a predetermined condition does not perform at least one activity selected from the group consisting of DNA replication, RNA transcription and protein synthesis.

16. A method according to claim 15 wherein the predetermined condition is selected from the group consisting of pH, temperature, composition of the substrate material and presence/absence of an inducer substance.

17. A method according to claim 1 wherein the culture comprises a bacterial strain which is capable of increasing the size of the cells without mitosis.

18. A modified lactic acid bacterium that is modified to become incapable of performing DNA replication, RNA transcription or protein synthesis in a specifically defined substrate material which is limited with respect to at least one compound that is required by the bacterial strain for DNA replication, RNA transcription or protein synthesis, said modified bacterial strain is capable of being metabolically active in said substrate material, whereby the strain is not susceptible to attack by bacteriophages, subject to the limitation, that the lactic acid bacterium is not a strain selected from the group consisting of strain DN101, DN102, DN103, DN104 and DN105 (DSM12289).

19. A lactic acid bacterium according to claim 18 wherein the bacterial strain is a mutant strain being auxothrophic in respect of a compound which is not present in the substrate material and which is required by the strain for replication.

20. A lactic acid bacterium according to claim 19 wherein the mutant strain is a *thyA* mutant including *Lactococcus lactis* strain MBP71 deposited under the accession number DSM12891.

21. A starter culture compostion comprising the lactic acid bacterium of any of claims 18-20.

22. A starter culture composition comprising a lactic acid bacterium obtainable by the method according to claim 1 in combination with at least one further lactic acid bacterium.

23. A composition according to claim 22 which further comprising at least on component enhancing the viability of the bacterial active ingrediant during storage including a bacterial nutrient or a cryoprotectant.

24. A method of manufacturing a food or feed product comprising adding a starter culture composition according to any of claims 21-23 to a food or feed product starting material and keeping the thus inoculated starting material under conditions where the lactic acid bacterium is metabolically active.

25. A method according to claim 24 wherein the food product starting material is milk.

26. Use of a culture as obtained in the method of claim 1 or a lactic acid bacterium according to any of claims 18-20 as a starter culture in the preparation of a product selected from the group consisting of a dairy flavour, a product for cheese flavouring, a food product and a feed product.

27. A method of preventing that a lactic acid bacterial starter culture is infected by bacteriophages in the manufacturing of a food or feed product, the method

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comprising adding as a starter culture a lactic acid bacterium obtained by the method according to claim 1 to a food or feed product starting material which is limited with respect to at least one compound that is required by the bacterial strain for DNA replication, RNA transcription or protein synthesis and keeping the thus inoculated starting material under conditions where the lactic acid bacterium is metabolically active, whereby, if the substrate material is contaminated with a bacteriophage, the metabolic activity of the bacterial culture is substantially unaffected by the bacteriophage.